# Ability of Janus Particle as Single Cell Sensor to Immobilize Plasma Cells and Collect Antibody

A Thesis
Presented to
The Academic Faculty

by
Ye Lim Lee

In Partial Fulfillment
of the Requirement for the Degree
B.S. in Biomedical Engineering with Research Option in the
College of Engineering

Georgia Institute of Technology
Fall 2018

Ability of Janus Particle as Single Cell Sensor
to Immobilize Plasma Cells and Collect Antibody

Approved by:

Dr. Todd Sulchek, Principal Investigator
School of Mechanical Engineering
*Georgia Institute of Technology*

Dr. S. Balakrishna Pai
School of Biomedical Engineering
*Georgia Institute of Technology*

Date Approved: 12/04/2018

**Acknowledgement**

I want to thank Dr. Todd Sulchek, professor of Georgia Tech ME department and my Research Mentor, and Katily Ramirez, PhD student, for allowing and leading me to conduct the research on the paper that is written on.

## Table of Contents

**Abstract**

The method to identify and isolate the proteins is one of the keys to the immunoassay production. However, currently there is no effective way to isolate the protein not only because of limited number of technologies for protein detection but also the existing technologies have downfall of low sensitivity for protein detection and the secreted protein cross-reactivity. The present paper demonstrates the isolation and collection ability of the Janus particle. The study demonstrates the creation of Janus particle with one side of the particle targets and binds plasma cells while the other side contains protein G that collects specific antibodies that are secreted. Experiments were conducted to determine the ability of 2 μm and 4 μm size Janus Particle. Moreover, the study was carried out with hybridoma cells to determine whether the Janus particle can bind to the plasma B cell and collect antibody with another hemisphere.

## Introduction

Proteins are key modulators for various functions in the human body. Cytokines, small proteins secreted from both immune and non-immune cells, are vital to cell signaling and are closely associated with immune system responses such as inflammation and central sensitization [1, 2]. The measurement of cytokine activities is extremely important because elevated or depressed concentration of cytokines are associated with inflammation or disease progression. Monitoring cytokine concentrations in body fluids can help to understand the disease pathogenesis and effects of treatment, and cytokine molecules can be utilized in several clinical applications such as cancer therapy and autoimmune disease treatments [3,4]. Antibodies, proteins secreted by plasma B cells in response to antigens, are key components of the immune system and are utilized in vaccine production, antibody therapy, disease diagnosis, and a wide variety of other biomedical applications. Isolating and purifying plasma B cells and their secreted antibodies specific to a target antigen is extremely important for the development of these applications, as well as analysis of different antibody molecules can provide further insight into how the immune system responds to different antigens.

Various techniques to quantify cytokines, such as immunoassay, ELISA, enzyme-linked immunosorbent spot (ELIspot), antibody array assay, and bead-based assay, have been used, but there are common challenges that undermine their effectiveness. Cytokines are often secreted into the extracellular environment in pM concentrations, and current detection techniques are not sensitive enough to detect such low concentrations accurately. In addition, interference from other heterophilic antibodies, rheumatoid factors, and cytokine-binding proteins creates noise in the measurements, and the dynamic process of cytokine secretion prevents accuracy and

reproducibility. ELISA produces the most reliable data but is time consuming (6 hours) and usually requires a relatively large sample volume (100 µL) [5].

The most standard technique to isolate antigen-specific B cells is fluorescence-activated cell sorting (FACS) after labeling surface antibodies with fluorescent antigen [6]. However, identifying and isolating plasma cells is an ambitious proposal because plasma cells lack surface antibodies and only constitute a tiny percentage of the whole blood cells [7]. A majority of diagnostic and therapeutic antibodies are produced using the mouse-human hybridoma technique which immortalizes antibody secreting cells and allows for the production of antibodies for a prolonged time period: however, this method is not suitable for human use and has created many controversies related to animal welfare [8, 9]. Therefore, to obtain antibodies for antigen that are specific for humans, it is best to collect antibodies directly from plasma B cells.

In the past 15 years, several advancements have been made for the detection and the quantification of cytokines and antibodies using magnetic beads, nanomaterials, antigen stimulation, and multiplex bead assays [5,10]. Each advancement aims to enhance sensitivity, selectivity, and/or simplicity, but all hold disadvantages such as high cost, high complexity, and limited availability of resources. High complexity compromises the survivability of cells, and high cost and limited availability prevents commercial use. Therefore, the development of a simpler yet effective protein detection and collection technique is pivotal for the efficient production of proteins and subsequent analysis, reproduction, and therapy.

This study proposes the utilization of bifunctional microparticles for the isolation of specific plasma B cells. Janus particles, particles with two physically and chemically distinct hemispheres, are engineered such that one side binds to antigen-specific plasma B cells and the other collects the secreted antibodies simultaneously. Anti-CD44 antibody conjugated on one

hemisphere targets the surface marker CD44 on plasma B cells, while protein G, which has been proven to have high antibody affinity, captures and immobilizes the antibodies [11]. The isolation efficiency of Janus particles is compared to that of mixed particles, which are protein G coated silica particles bifunctionalized with the conjugation of anti-CD44 antibodies without any spatial segregation. The team also investigates the relationship between particle size and the antibody collection efficiency to identify the best particle size for optimized cell isolation. Fewer steps involved in the proposed technique will enhance the survival rate of plasma cells and increase the isolation efficiency. Moreover, optimization of this technique will establish a new mechanism to identify and sort large numbers of specific plasma cells for further applications. The sorted plasma cells can be immortalized for mass production of antibodies, enabling the development of new antibodies for prevention and/or treatment of infectious diseases. Bifunctional microparticles hold many possibilities as the new novel cell isolation tool, and a successful development of this technique will benefit many applications in the biomedical and pharmaceutical fields.

## Methods and Materials

### Material

Silica microspheres with diameters of 4μm carboxylated polystyrene microspheres were purchased from Bangs Laboratories (Fishers, IN). Coupling reagents, including (3-Aminopropyl)triethoxysilane (APTES), 1-Ethyl-3-(3-dimethylaminopropyl)carbodiimide (EDAC), acetone, phosphate buffered saline (PBS), PolyLink Wash/Storage Buffer, PolyLink Coupling Buffer, Dulbecco's Modified Eagle's Medium (DMEM), Iscove's Modified Dulbecco's Medium (IMDM), gentamicin, penicillin/streptomycin, L-glutamine 200mM, 1% sodium pyruvate, concanavalin A Alexa Fluor 488 were purchased from Sigma-Aldrich (St. Louis, MO), fetal bovine serum from Atlanta Biologicals (Atlanta, GA). Bovine serum albumin (BSA) were purchased from Sigma-Aldrich (St. Louis, MO). Thiol-poly(ethylene glycol)-biotin, a heterobifunctional PEG derivative, was purchased from Nanocs (New York, NY). Human Bcl6 peptide were purchase from Abcam (Cambridge, UK). APC Anti-mouse/human Anti-CD44 antibody from Biolegend (San Diego, CA). Lightning-Link® Streptavidin from expedeon (San Diego, CA). Protein G was purchased from Protein Specialists (East Brunswick, NJ).

### Method

#### *Cell Culturing*

TIB 147 cells from ATCC (Manassas, VA) and BCL6 hybridomas from Iowa University (Iowa University Hybridoma Bank, Iowa City, IA) were used to test the particle's ability to collect and identify specific antibodies, anti-concanavalin A and anti-BCL6, from cell culture media and supernatants. Both cells were cultured at a concentration of $10^6$ cells/mL and incubated in humified incubator at 37°C with 5%. BCL6 hybridoma was cultured using IMDM

complete media with 20% fetal bovine serum, 1% L-glutamine 200mM, 1% sodium pyruvate 100Mm, 0.1% gentamicin 50mg/ml, and 0.1% penicillin/streptomycin.

*Preparation of Janus Particle*

The protocol to create the Janus particle was modified based on proposed mechanism by Tang et al [12]. The 2 and 4μm silica particles are washed multiple times using 100% ethanol in 1:10 ratio of particle stock solution to ethanol. After two wash, the particle is kept in the ethanol solution in chosen ratio for approximately 5minutes then 8 μL was depostied onto the glass slide, which was moved onto orbital rotator at 200rpm to completely dry. Then, the tape is placed in opposite side of the deposition layer, and the slides were coated with a layer of gold following a titanium adhesion layer using a metal evaporation process (CHA E-Beam Evaporator). The thickness of titanium and gold deposition was in 0.1 kÅm, 0.1kÅ and 0.5kÅ and 1 kÅ with a rate of 1 Å/s, respectively, for 2μm and 4 μm. After the gold deposition, the slide was placed into 50 mL tube filled with deionized water and sonicated for 5 minutes. After the particle is collected from the tube, the gold hemisphere is modified using thiol-PEG-biotin, and silica side with APTES and the carboxylated polystyrene via EDAC.

## Result

*Janus particle with two hemispheres targeting the cell*

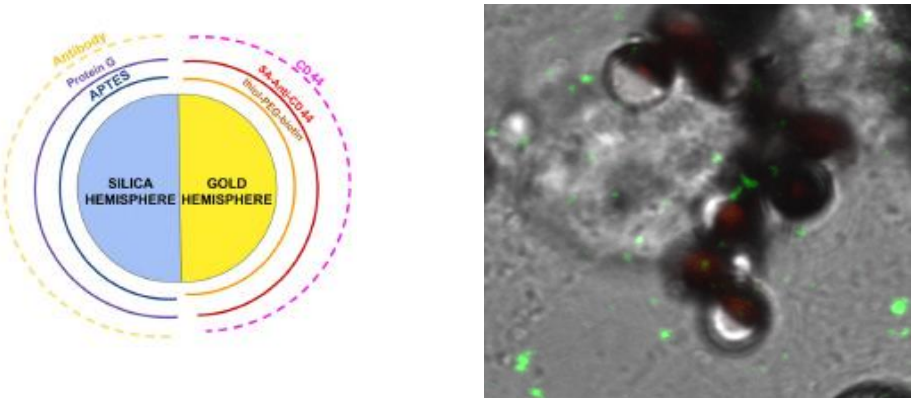


**Figure 2. The Visual representation of Janus Particle (on left) and the Confocal Laser Microscopy image of Janus Particle of 3.98 µm targeting the BCL6 hybridoma cells (on right).**

The visual representation on the Figure 2 (left) represents formation of Janus particle that is used to target the specific cell, BCL6 hybridoma cell(Figure 2 right). The Figure 2 image was used to confirm that Janus particle has two distinct hemispheres and demonstrate that Janus particle is accurately produced as desired with both sides precisely functionalized with bifunctionalized surface spatial segregation. The black slide on the image represents the gold hemisphere and white slide correspond to silica side. The gold hemisphere targets the specific cells and silica hemisphere collect the antibodies secreted by the cells. The figure also shows that the particles can identify the specific cells.

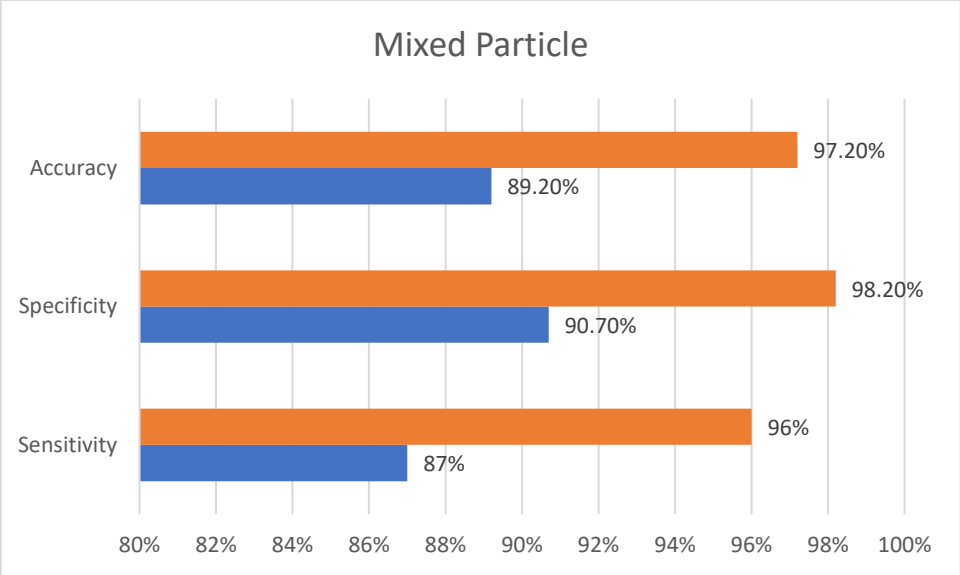


**Figure 3. Accuracy, Specificity, and Sensitivity of the Mixed particles, Orange bar representing 4µm and Blue bar representing 2µm based on the result collected based on confocal analysis and flow cytometry result.**

Based on Figure 3, the percentages of all three categories for both 2µm and 4µm Janus Particles are all above the 85% indicating that within the mixture of hybridoma cells, the bifunctional particles are able to detect the cell that secretes a specific antibody with the high sensitivity, specificity, and accuracy. However, in comparison, the particle of 4µm demonstrated higher percentage in all three criteria compared to 2µm particle.

## Discussion

The Janus demonstrated the ability to isolate specific hybridoma cells and to collect secreted antibodies. These properties were confirmed using fluorescent dye on cells as well as labeling on secreted antibodies. The binding properties was augmented based on the anti-CD conjugation resulted in higher specificity on isolating the cells secreting the antibodies.

Both 2μm and 4μm Janus Particles showed the ability in isolating single cell and collecting the antibody, they showed high ability in different aspects with all the percentage above 85%. Comparing the ability of 2μm, 4μm Janus particle demonstrated greater ability in overall all the aspects in terms of accuracy, specificity, and sensitivity, which indicates that 4μm Janus particle demonstrated better both targeting and collection ability as well as the localization of antibody collection. The 4μm Janus particle with greater surface area of distinct hemisphere could enhance the binding to cell and collection of antibodies. The Janus particle is showing great potential as a single cell sensor and protein collector.

## Conclusion

The Janus particle that demonstrates promising results as a novel biotechnology application to study various protein secreting cells and to collect the secretions. A secreted products reagent has potential advantage for biomedical related research in terms of isolation of rare cells or genetic variants of cytokine-secreting lymphocytes, or single-cell analyses of exocytosis, hormones, or viruses. Moreover, these collected proteins and antibodies could be potentially applicable to enhance currently existing therapies by selecting only specific cells. To further enhance the study and investigate greater use of the bifunctional particles in aspect of vaccine development and disease therapy.

**Citation**

[1] Stenken, J. A., & Poschenrieder, A. J. (2015). Bioanalytical chemistry of cytokines – A review. Analytica Chimica Acta, 853, 95-115. doi:https://doi.org/10.1016/j.aca.2014.10.009

[2] Zhang, J.-M., & An, J. (2007). Cytokines, Inflammation and Pain. International anesthesiology clinics, 45(2), 27-37. doi:10.1097/AIA.0b013e318034194e

[3] Steinke, J. W., Illei, G., Uzel, G., & Mican, J. M. (2008). 94 - Cytokine therapy A2 - Rich, Robert R. In T. A. Fleisher, W. T. Shearer, H. W. Schroeder, A. J. Frew, & C. M. Weyand (Eds.), Clinical Immunology (Third Edition) (pp. 1393-1404). Edinburgh: Mosby.

[4] Catalfamo, M., Le Saout, C., & Lane, H. C. (2012). The role of cytokines in the pathogenesis and treatment of HIV infection. Cytokine & Growth Factor Reviews, 23(4), 207-214. doi:https://doi.org/10.1016/j.cytogfr.2012.05.007

[5] Liu, G., Qi, M., Hutchinson, M. R., Yang, G., & Goldys, E. M. (2016). Recent advances in cytokine detection by immunosensing. Biosensors and Bioelectronics, 79, 810-821. doi:https://doi.org/10.1016/j.bios.2016.01.020

[6] Kodituwakku Aruna, P., Jessup, C., Zola, H., & Roberton Don, M. (2003). Isolation of antigen-specific B cells. Immunology and Cell Biology, 81(3), 163-170. doi:10.1046/j.1440-1711.2003.01152.

[7] Branam, G. E., & Paff, J. R. (1975). Plasma Cells and Plasmacytoid Lymphocytes in the Peripheral Blood. Laboratory Medicine, 6(11), 24-30. doi:10.1093/labmed/6.11.24.

[8] Osherovich, L. (2010). Making mAbs en masse. Nature, 3. doi:10.1038/scibx.2010.37.

[9] Committee on Methods of Producing Monoclonal Antibodies Institute for Laboratory Animal Research National Research Council, "Monoclonal Antibody Production," NATIONAL ACADEMY PRESS , Washington, DC , (1999).

[10] Pinder, C. L., Kratochvil, S., Cizmeci, D., Muir, L., Guo, Y., Shattock, R. J., & McKay, P. F. (2017). Isolation and Characterization of Antigen-Specific Plasmablasts Using a Novel Flow Cytometry-Based Ig Capture Assay. J Immunol, 199(12), 4180-4188. doi:10.4049/jimmunol.1701253.

[11] Jackson, A. J., Karle, E. M., & Hage, D. S. (2010). Preparation of high-capacity supports containing protein G immobilized to porous silica. *Analytical Biochemistry, 406*(2), 235-237. doi:10.1016/j.ab.2010.07.004

[12] H. F. Liu, "Recovery and purification process development for monoclonal antibody production," *landesbioscience,* vol. 2, no. 5, pp. 488-499, 2010.